Evaluation of Mirex, Photomirex and Chlordecone in the Terrestrial Aquatic Laboratory Model Ecosystem

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The insecticides mirex and chlordecone and the mirex photodegradation product, photomirex, were evaluated in a terrestrial aquatic laboratory model ecosystem. Although chlordecone was to some extent degraded during the 33 days, neither mirex nor photomirex produced identifiable levels of decomposition products in the water or in any of the organisms of the model ecosystem. All three compounds accumulated significantly in the organisms of the model ecosystem, with chlordecone the least bioaccumulative and photomirex the most bioaccumulative.

Introduction

The insecticide mirex [1,1a,2,2,3,3a,4,5,5,5a,-5b,6-dodecachloroocta-1,3,4-metheno-1Hcyclobuta (cd) pentalene; CA Registry No 2385-85–5] is a stomach poison with little contact toxicity, used primarily in baits against the imported fire ant [Solenopsis invicta Buren] in the southern United States. Between its introduction in 1962 and its banning in 1976, mirex was sprayed aerially over millions of acres. Despite the relatively low levels required (4.2 g/ha, or 1.7 g/A), a total of 800,000 lb of mirex was used against fire ants in those years (1). Its extreme environmental persistence (2,3) led to measurable contamination of fish and wildlife (4,5), aquatic invertebrates (6), and humans (7) in treated areas. Most of the public debate about mirex, and most of the concern over its environmental effects, have resulted from its use as an insecticide (1,8–10). Nevertheless, insecticidal use accounted for only 26% of the mirex produced by Hooker Chemicals and Plastics Corp. before 1977: the other 74% consisted of the same product, marketed as a flame retardant under the name Dechlorane (1). In either guise, mirex is extremely resistant to degradation in the environment or by higher organisms (1,11-13), although some degradation occurs (14). Mirex

manufacture has also resulted in environmental contamination. Near the manufacturing site of mirex on the Great Lakes, fish contain measurable body burdens not only of mirex but also of its degradation products (15).

Among the few known degradation products of mirex are chlordecone [Kepone, 2-ketomirex, or 1,1a,3,3a,4,5,5,5a,5b,6-decachlorooctahydro-1,3,4-metheno-2H-cyclobuta (cd) pentalen-2-one, CA Registry No. 143-50-5]; and photomirex [1,1a,2,2,3,3a,4,5,5,5a,5b-undecachlorooctahydro-1,3,4-metheno-1H-cyclobuta (cd) pentalene, CA Registry No. 39801-14-4]. Photomirex is not a commercial product, but chlordecone was marketed as an insecticide until 1976, when severe health and environmental effects were identified at its production site in Hopewell, VA (16). Photomirex has been identified as a contaminant of fish in Lake Ontario, presumably as a result of mirex production at Niagara Falls, NY (1) and subsequent exposure of the mirex to UV light (17). In addition to their close chemical relationship, both mirex and chlordecone are highly lipophilic, highly persistent, and carcinogenic (3,18-20). Given their persistence, both mirex and chlordecone will remain in the natural ecosystems which they have contaminated for the foreseeable future. One attempt to minimize environmental contamination by mirex was to formulate it with aliphatic amines and ferrous chloride to facilitate photodegradation (9,10). This formulation, known as ferriamicide, presupposes the manufacture of mirex, and so does not affect the

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environmental effects occurring as a result of mirex manufacture for either insecticidal or flame retardant uses. However, in light of the continuing controversy over use of mirex and its formulated analog, ferriamicide (9), as well as the nonpesticidal uses of this organochlorine compound, it was pertinent to compare the behavior of mirex, chlordecone, and photomirex in a laboratory model ecosystem in order to assess their behavior, bioaccumulation, and persistence under standardized conditions.

The terrestrial aquatic laboratory model ecosystem is a sensitive laboratory tool which can quickly provide answers to critical questions about the environmental behavior of xenobiotics. Consisting of sand, water and a few organisms, the model ecosystem most closely simulates a farm pond surrounded by fields under cultivation. The use of sand minimizes absorption while maximizing leaching: such use of limit conditions is a desirable simplification in model systems. Similarly, the constant 26°C temperature and simulated solar lighting maximize both photodecomposition and biodegradation. The organisms (plants, algae, caterpillars, two invertebrates and one vertebrate) provide several food chains, yet sufficiently few species are used to permit analysis of the sequential transport of the pesticide as a more complex system might not.

The laboratory model ecosystem has been designed to evaluate the fate of pesticides in water, and in particular to measure the potential of a given compound for ecological magnification as it progresses through a food chain.

Materials and Methods

The laboratory model ecosystem evaluations were carried out in 10-gal glass aquaria with a sloping terrestrial-aquatic interface of pure white sand, as previously described (21,22). The ¹⁴C-radiolabeled compounds were applied from acetone solution to Sorghum vulgare seedlings grown in the terrestrial portion. Levels of treatment were 1.0 mg/system (or approximately 0.2 kg/ha) for mirex, photomirex and chlordecone. A supplemental study of mirex was made by using a 5.0 mg/system (approximately 1.0 kg/ha). The treated leaves were eaten by fourth instar saltmarsh caterpillars (Estigmene acrea), whose activities and fecal products contaminated the aqueous portion of the system.

The radiolabeled products were transferred through several aquatic food chains, e.g., alga, $(Oedegonium\ cardiacum) \rightarrow snail\ (Physa\ sp.); \rightarrow plankton \rightarrow water\ flea\ (Daphnia\ magna) \rightarrow mos-$

quito (Culex pipiens quinquefasciatus) \rightarrow fish (Gambusia affinis). After 33 days in an environmental chamber at 26.5°C and a 16-hr photoperiod at 5,000 ft-candles simulated daylight, the organisms were extracted with acetonitrile and the extracts concentrated to a few milliliters, which were then spotted on thin-layer chromatography (TLC) plates containing fluorescent marker (Silicar 7GF, 250 µm). Thin-layer chromatography was carried out in chloroform, and the developed TLC plates exposed to X-ray film. The location of parent compound and metabolites was determined from the film after development, and the corresponding areas of the TLC plates were scraped into scintillation fluid and counted in a liquid scintillation counter, using cocktail D (5 g PPO and 100 g naphthalene in dioxane to make one liter). Counts were corrected to dpm by using the channels ratio quenching correction. The absolute (microgram) quantities of parent compound and metabolites in each organism and in water are calculated from the specific activity of the applied compound and the dpm per spot on the TLC plate. The relative (parts per million) concentration of parent compound and metabolites was determined from the microgram pesticide equivalents in the extracts and the weight of the organism from which they derive. The concentrations of parent compound in each organism and in water are then used to calculate the ecological magnification (EM):

 $EM = \frac{\text{concentration of parent compound in organism}}{\text{concentration of parent compound in the water}}$

and the biodegradation index (BI):

 $BI = \frac{concentration of polar metabolites}{concentration of nonpolar metabolites}$

The residues remaining after extraction were counted by total combustion to ¹⁴C-CO₂ by the Packard TriCarb sample oxidizer to determine the unextractable radioactivity.

The model ecosystem analysis focuses on the relative levels of parent compound and metabolites in water vis a vis their relative levels in the aquatic organisms. There is no attempt to establish the total quantitative distribution of all the applied ¹⁴C, a considerable fraction of which is retained in the sand of the ecosystem; in the caterpillars which serve to transport the chemical from the plants into the water; and in the plants themselves, especially in the roots, which are never eaten by the caterpillars. More pertinent to the question of environmental fate is the removal

from water of chemical which enters the water. Therefore, the percent of radiolabel in solution which is recovered from the organisms is calculated from the maximum levels of ¹⁴C in the water and from the levels of ¹⁴C in the organisms at the time the system is terminated.

Mirex, ¹⁴C-ring-labeled, 1.74 mCi/mmole, was obtained from Mallinkrodt Chemical Co. It had a radiopurity of 98%. Chlordecone-¹⁴C ring-labeled, 17.4 mCi per mmole, 98% radiopurity, was obtained as a gift from Allied Chemical Co. Photomirex, ¹⁴C-ring-labeled, 0.51 mCi/mmole, 96% radiopurity, was a gift of Dr. I. Chu, then of Environment Canada.

Results and Discussion

Table 1 shows the movement of radioactivity from plants into the water of the ecosystem, given as ppm pesticide equivalent, since total ¹⁴C was counted in water samples taken during the course of the ecosystem. The data in Tables 2-4 represent the quantitative distribution of the ¹⁴C in the spots on the radioautographs which were made from extracts of organisms. The results are given in ppm, calculated from the dpm per spot and the weight of the organisms from which the extract was derived. Because of their extremely low solubility (0.085 ppm for mirex), water levels of both mirex and photomirex remained very low throughout the 33 day period. Water levels for chlordecone include the degradation compounds formed by this chlorinated hydrocarbon insecticide as well as levels of parent compound. That

Table 1. Levels of C¹⁴ in the waters of a laboratory model ecosystem over a 30-day period, given in ppm pesticide equivalents.

DAY	¹⁴ C, ppm pesticide equivalents						
	Mirex	Photomirex	Chlordecone				
1	0.0205	0.0200	0.0122				
2	0.0114	0.0412	0.0170				
3	0.0149	0.0256	0.0200				
5	0.0299	0.0228	0.0254				
7	0.0183	0.0603	0.0269				
14	0.0201	0.0335	0.0464				
21	0.0105	0.0367	0.0409				
26	0.0237	0.0258	0.0192				
29	0.0179	0.0399	0.0238				
33	0.0182	0.0087	0.0238				

degradation products do not contribute significantly to the total radioactivity in the mirex and photomirex ecosystems is shown in Tables 2 and 3. Table 4 shows the corresponding data for chlordecone, which was to some extent degraded during the course of the study. Table 5 summarizes the potential of mirex, photomirex and chlordecone for bioaccumulation in food chains, showing the ecological magnification of each in the algae, snails, mosquitoes and fish, and their biodegradability in the same organisms.

The percent recoveries in the various organisms of the total ¹⁴C-mirex, photomirex and chlordecone applied to the model ecosystems are shown in Table 6. The total recoveries from water and organisms at the completion of the ecosystem range from 9.33 to 19.51% of the amounts applied. The balance remains in the sand substrates as well as in the caterpillars, which ate the treated

Table 2. R_f values and amounts, of mirex and its degradation products found in the water and organisms of a model ecosystem.^a

	R_f^{b}	${ m H_2O}$	Oedegonium (alga)	Physa (snail)	Culex (mosquito)	Gambusia (fish)
Extractable						
¹⁴ C (total), ppm		0.0180	9.70	18.40	13.60	3.50
Mirex, ppm	0.95	0.0157	9.49	18.29	13.54	3.45
Polar, ppm	0.00	0.0023	0.21	0.11	0.06	0.05

 ${\tt a14C-ring-labeled~1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachloroocta-1,3,4-metheno-1H-cyclobuta~(cd)~pentalene.} \\ {\tt bSilicar~7GF,~250~microns;~chloroform.}$

Table 3. R_f values and amounts of photomirex and its degradation products in the water and organisms of a model ecosystem.^a

	R_f b	${ m H_2O}$	Oedegonium (alga)	Physa (snail)	Culex (mosquito)	Gambusia (fish)
Extractable						
¹⁴ C (total), ppm		0.0080	0.5699	15.0990	6.5166	2.7653
Photomirex, ppm	0.91	0.0041	0.5454	14.7970	6.2299	2.5883
Polar, ppm	0.00	0.0039	0.0245	0.3020	0.2867	0.1770
Unextractables, ppm		0.0007	0.5801	0.9878	0.2980	2.3686

 a_{14} C-ring-labeled 1,1a,2,2,3,3a,4,5,5a,5b-undecachlorooctahydro-1,3,4-metheno-1H-cyclobuta (cd) pentalene. b_{11} Silicar 7GF, 250 microns, chloroform.

Table 4. R_f values and amounts of chlordecone and its degradation products in the water and organisms of a model ecosystem.

	R_f^{b}	H ₂ O	Oedegonium (alga)	Physa (snail)	Culex (mosquito)	Gambusia (fish)
Extractable						
14C (total), ppm		0.0219	0.0102	20.2717	15.5756	3.7585
6	0.87	_	_	0.0750	_	
5	0.81	_	0.0007	0.1257	0.1184	0.0033
4	0.66	0.0001	0.0001	_	_	
3	0.63		_	0.0649		_
2	0.44			0.0689		_
1	0.34	trace	0.0005	0.7683	0.0862	_
Chlordecone, ppm	0.13	0.170	0.0060	10.8352	8.6180	2.0037
Polar, ppm	0.00	0.0048	0.0029	0.3337	0.8408	1.7515
Unextractables, ppm		0.0019	0.7330	0.9823	3.1442	3.1528

 $^{^{}a14}$ C-ring-labeled 1,1a,2,3a,4,5,5,5a,5b,6-undecachlorooctahydro-1,3,4-metheno-1H-cyclobuta (cd) pentalene-2-one. b Silicar 7GF, 250 microns, chloroform.

Table 5. Ecological magnification (EM) and biodegradation index (BI) of mirex, photomirex and chlordecone in the organisms of the terrestrial-aquatic model ecosystem.

Ecological magnification (EM)				Biodegradation index (BI)			
Chlordecone	Mirex	Photomirex	Species	Chlordecone	Mirex	Photomirex	
0.35	604	133	Oedegonium	0.40	0.022	0.04	
637.4	1165	3609	Physa	0.03	0.006	0.02	
506.9	862	1519	Culex	0.10	0.004	0.05	
117.9	219	631	Gambusia	0.10	0.004	0.05	

sorghum. A more important aspect is the total recovery in the various organisms of the ¹⁴C which reached the water and was subsequently lost from the water due to uptake by the various aquatic organisms (Table 6). This was highest in the fish Gambusia at the top of the food chain, and ranged from approximately 8 to 12%.

At the termination of the ecosystem, undegraded chlordecone was found at 10 ppb in the water, but had bioaccumulated to 2 ppm in fish during the 3 days the fish were in the system (Table 4). Snails contained even higher levels, 10.8 ppm of chlordecone. Nevertheless, some degradation of chlordecone occurred, and metabolites were seen in every organism of the ecosystem (Table 4). In the organisms, the percent of extractable radioactivity which was attributable to

chlordecone (rather than to degradation products) was never over 60% (Table 5). In water, however, chlordecone accounted for 78% of the total extractable ^{14}C .

A portion of the data from the mirex ecosystem was published previously (23). No metabolites were present except as polar and unextractable compounds (Table 2). Mirex predominated in all the organisms, with 98.6% of the radiolabel in fish and 99.4% of the radiolabel in snails attributable to mirex. Moreover, despite the high light and temperature levels, no mirex metabolites, other than the small amounts of radioactivity in the polar fraction, were seen. The EM values for mirex were 219 in fish and 1597 in snails (Table 2), significantly greater than the corresponding EM values of 118 and 637 seen for chlordecone

Table 6. Biomass recovery of xenobiotics from organisms and water of model ecosystems.

		¹⁴ C recovered, %					
	recovery, %	In alga	In snail	In mosquito	In fish	Total	
Mirex	14C lost from H ₂ O	12.72	1.10	0.22	7.66	21.71	
	Total 14C applied	1.041	0.09	0.018	0.63	1.78	
	Total 14C recovery, was						
Photomirex	14C lost from H ₂ O	3.61	0.778	0.12	12.39	16.90	
	Total 14C applied	0.82	1.77	0.027	2.82	3.84	
	Total 14C recovery, was	ter and organisms	s: 9.93%				
Chlordecone	¹⁴ C lost from H ₂ O	5.61	0.54	0.19	11.66	18.00	
	Total 14C applied	0.88	0.085	0.030	1.85	2.85	
	Total 14C recovery, was	ter and organisms	s: 19.51%				

(Table 4). These values agree very well with a second model ecosystem evaluation from this laboratory that found EM values for mirex of 198 in fish and 1597 in snail (unpublished).

Qualitatively similar results were obtained by Huckins et al. (24) from studies with fathead minnows, in which no mirex metabolites were observed, and which continued to accumulate mirex without reaching plateau levels over a 56day period. In the same studies, chlordecone was recovered at very low levels from the minnows, but appeared to bind covalently to lipids in the fish, leading to unidentified ¹⁴C-labeled compounds. These compounds may be similar to the unidentified compounds seen in the chlordecone ecosystem (compounds 1-6, Table 4), which were also more lipophilic than chlordecone itself. This may be an artifact, however, as chlordecone readily forms a monohydrate with a water solubility of about 0.6%

Despite the absence of identifiable dechlorinated metabolites of mirex in the ecosystem, there is evidence from laboratory and field studies that, over much longer periods of time, photomirex and/or dihydromirex will result from exposure of mirex to UV light. Persistence and bioaccumulation of photomirex have also been documented. In the laboratory model ecosystem, under the high light intensity of the plant growth chamber, photomirex was persistent, did not generate intermediate metabolites, and accumulated significantly in all the organisms, with EM equal to 631 in fish and 3609 in snails (Table 5). While the ecological magnification of photomirex was of the same order as that of mirex (Table 5), the percent of extractable ¹⁴C recovered as parent compound ranged from 94% in fish to 98% in snails (Table 5). It should be emphasized that high levels of extractable radioactivity from radiolabeled xenobiotics in organisms of the model ecosystems are characteristic of very stable, lipid-soluble environmental pollutants such as DDT, DDE and the polychlorinated biphenyls, and that this parameter is of key significance in evaluating model ecosystem studies (25,26).

The significance of these data becomes apparent in the light of the known human health hazards of chlordecone (27–29) and the teratogenic and carcinogenic effects of mirex (3,19,30,31). Although no specific human health hazards have been attributed to photomirex, this is more probably a function of the close geographic correspondence between mirex and photomirex pollution, and of the general difficulty of assessing the effects of low level pollutants, than of the innocuous nature of photomirex (31–34). Since the worst

environmental pollution by photomirex occurs as a consequence of the manufacture of mirex rather than during the use of mirex as a pesticide, the proposed substitution of ferriamicide for mirex in order to facilitate photodegradation (10) will not effectively diminish photomirex contamination. The persistence, ecological magnification, and extreme resistance to degradation of photomirex make it a major environmental hazard.

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